Mosquito surveillance 2004

B Cahoon-Young, Manager, CVEC Arbovirus Lab
A Hom, VBDS, DHS
WK Reisen, BSL3 Committee, CVEC, &
Vectorborne Disease Committee, MVCAC

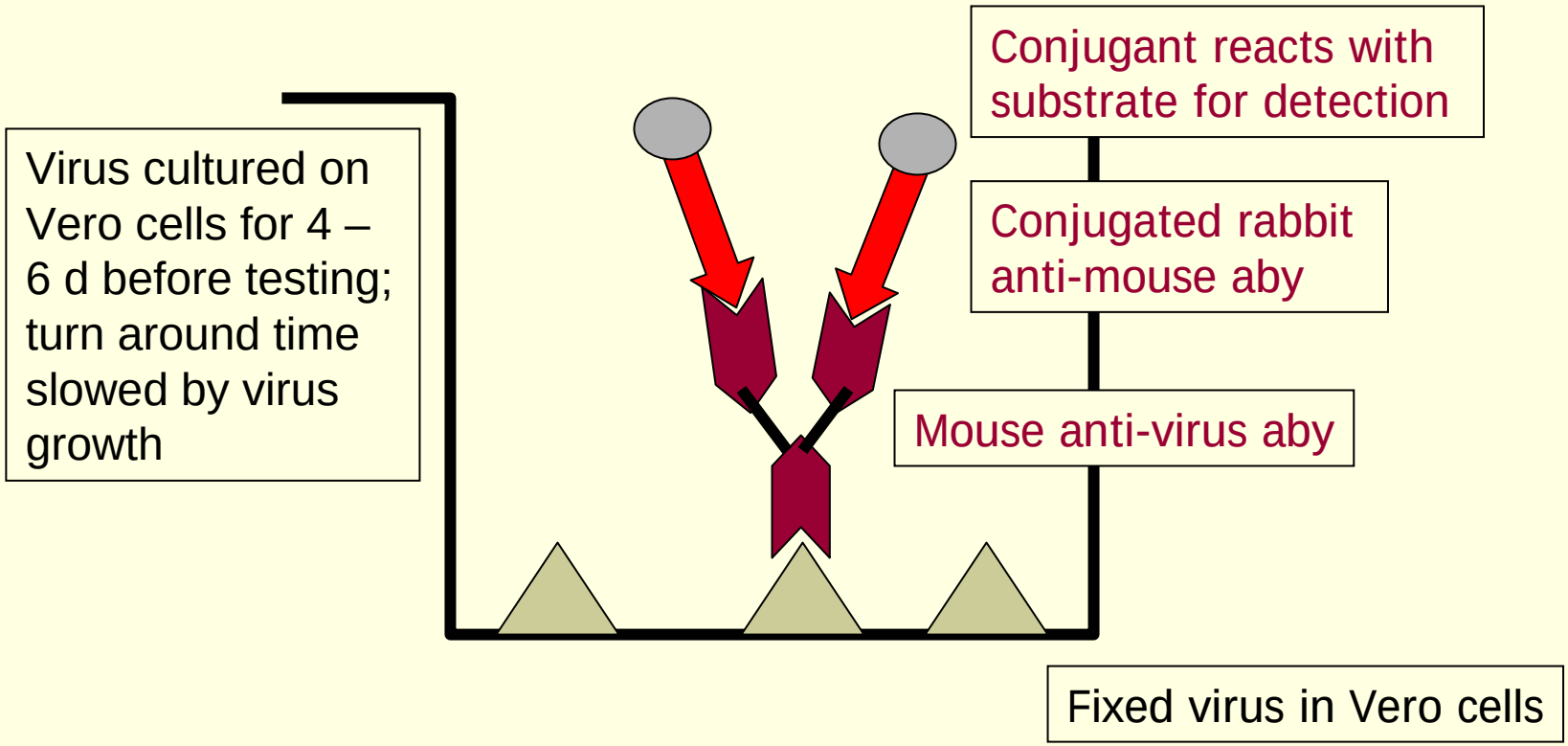
CVEC Arbovirus Unit Technical Staff

Not shown are Student Helpers: Thuan, Sean and Davis

Topics covered

- Transition from in situ EIA to multiplex RT-PCR
- Semi-automated testing system
- Data reporting
- Results – 2004
- Data utilization: MIRs, Risk Model
- Comparison to VecTest/RAMP
- Testing protocol for 2005

In situ enzyme immunoassay [EIA] - 2003



Molecular methods - 2004

- Fast:
  - RNA extraction ca. 3 h
  - RT-PCR ca. 3 h
  - Able to multiplex [test for > 1 virus at a time]
- Semi-automated; 87 samples per ‘run’
- Sensitive: range 1 – 5 PFU*
- Quantitative: can relate virus PFU to Ct** scores

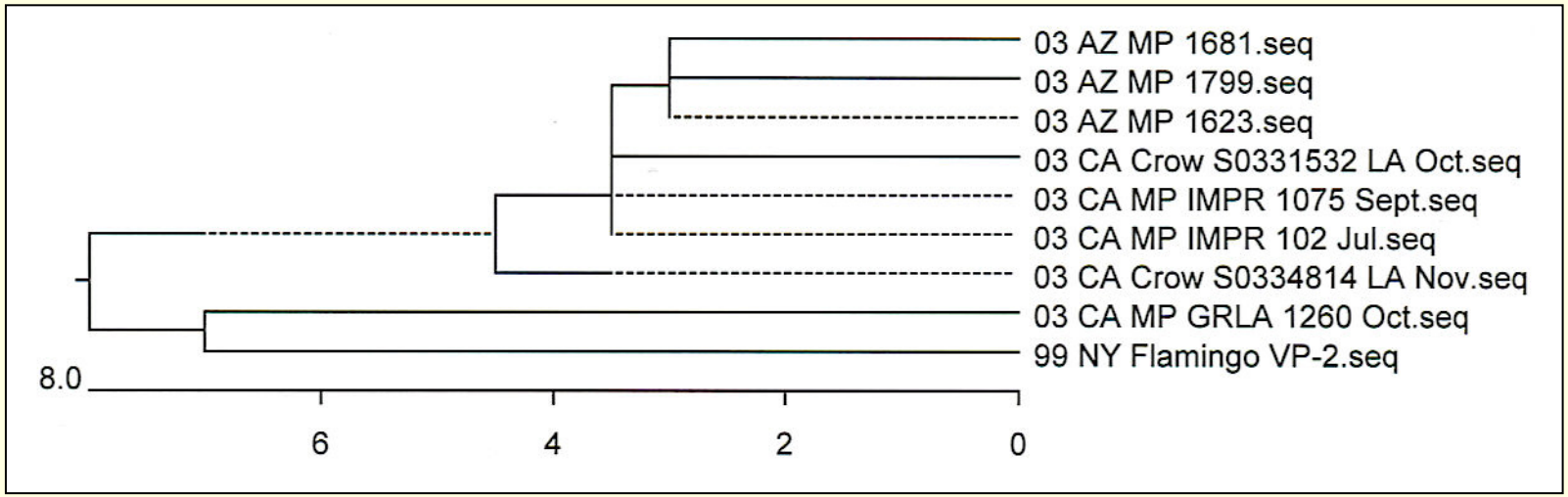
*PFU = plaque forming units of virus

**Ct = number of thermocycles until specimen positive

Specimen flow and capacity 2004

- Turn around time for pools ranged from 7-10 d
- Tested ca. 550 pools/wk [max 646] during July-August [never exceeded capacity of 800 pools/wk]
- Apr – June [method transition period]:
  - Tested *Ochlerotatus* for CEV
  - Confirmed all WNV multiplex RT-PCR positives by singleplex RT-PCR and in situ EIA.
- After July [stream-lined paradigm for throughput]:
  - Discontinued CEV testing
  - Discontinued confirmation of *Cx. tarsalis* and *Cx. p. quinquefasciatus* positives from positive areas

RT-PCR primer selection: WNV



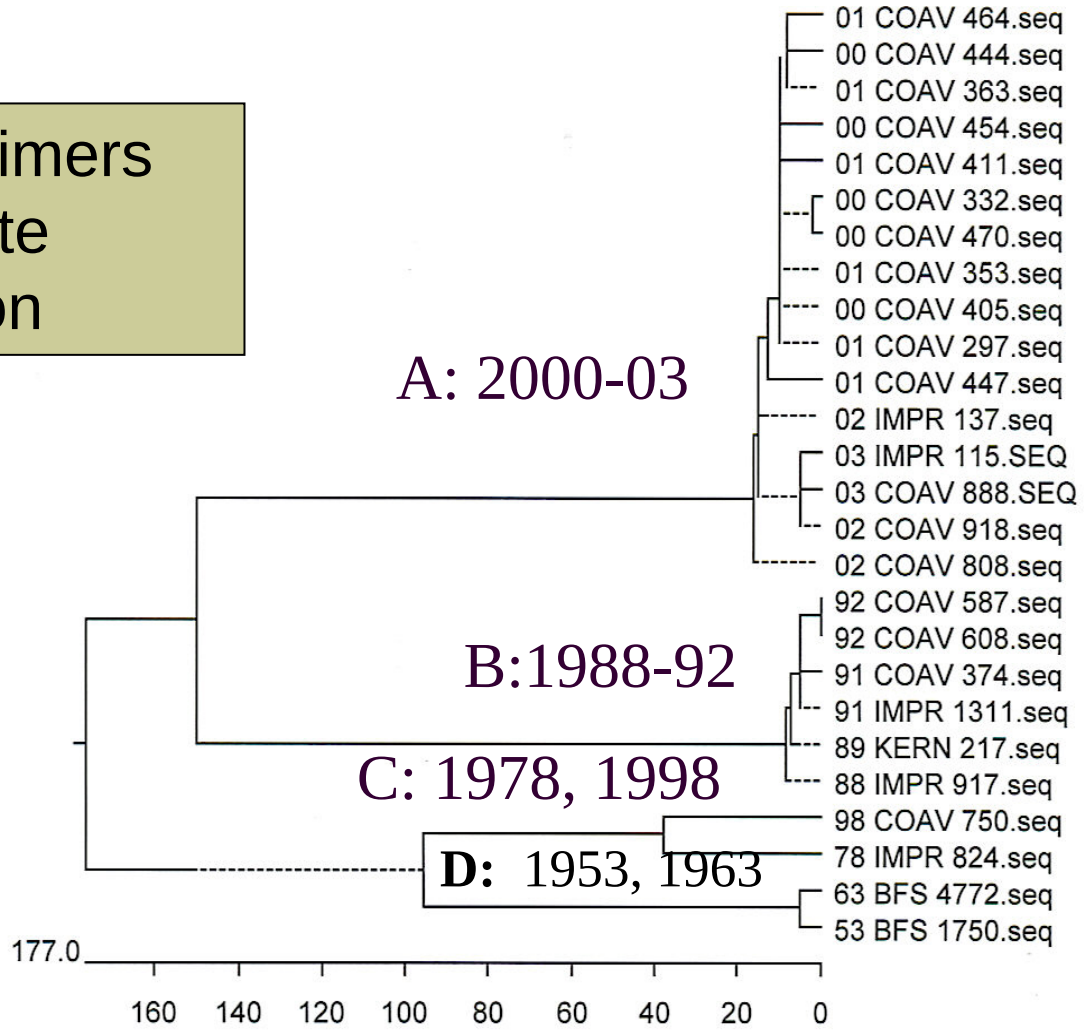
Minimal change among WNV isolates from mosquitoes and birds in CA and AZ.

Can use available primer sets.

Unpubl data from: Brault & Green, 2004

Genetic differences among strains of SLEV isolated from Coachella and Imperial Valleys, 1978-2001

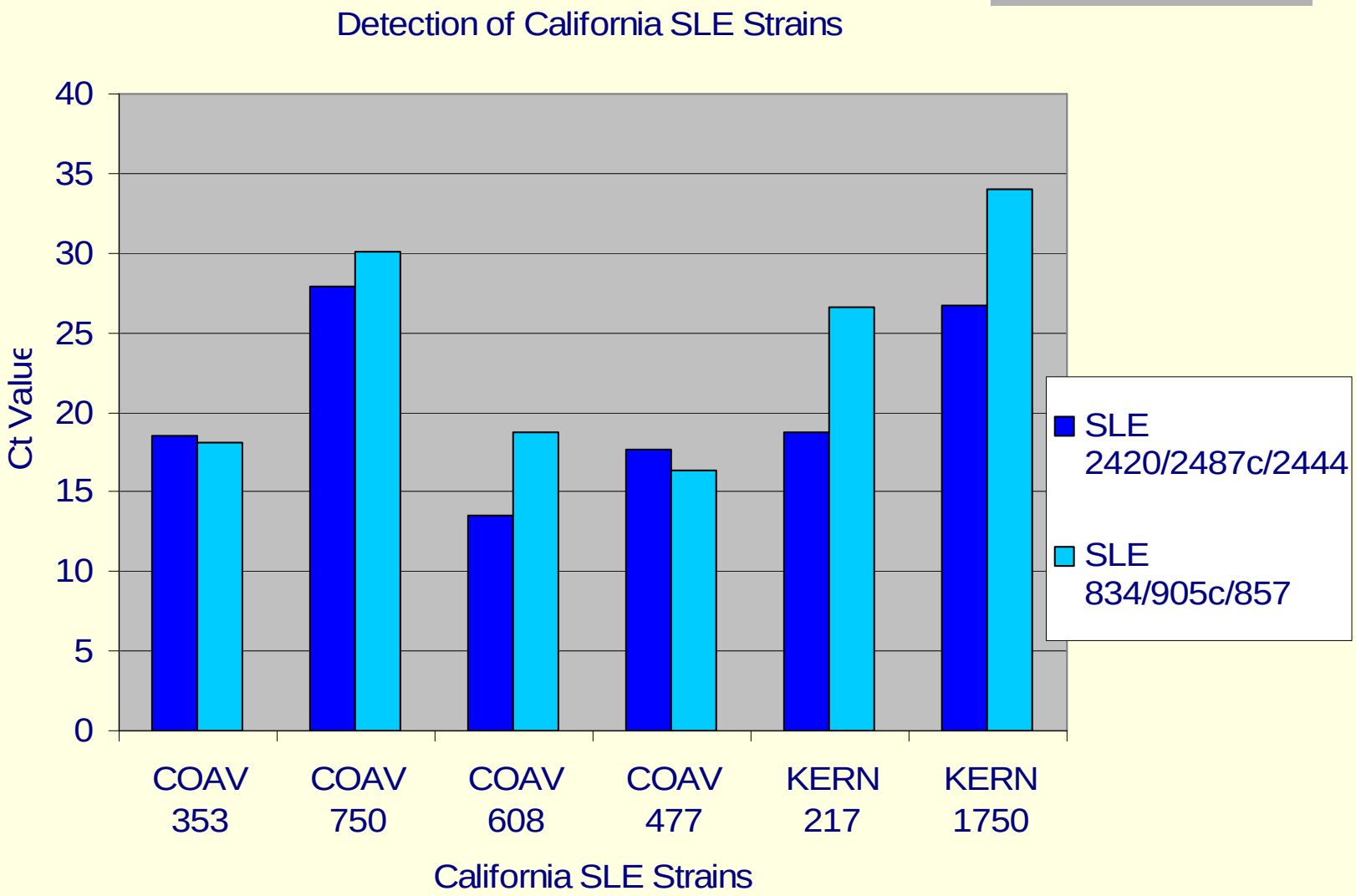
Must design primers to accommodate genetic variation



Updated from Reisen et al. 2001 by Brault & Green, 2004

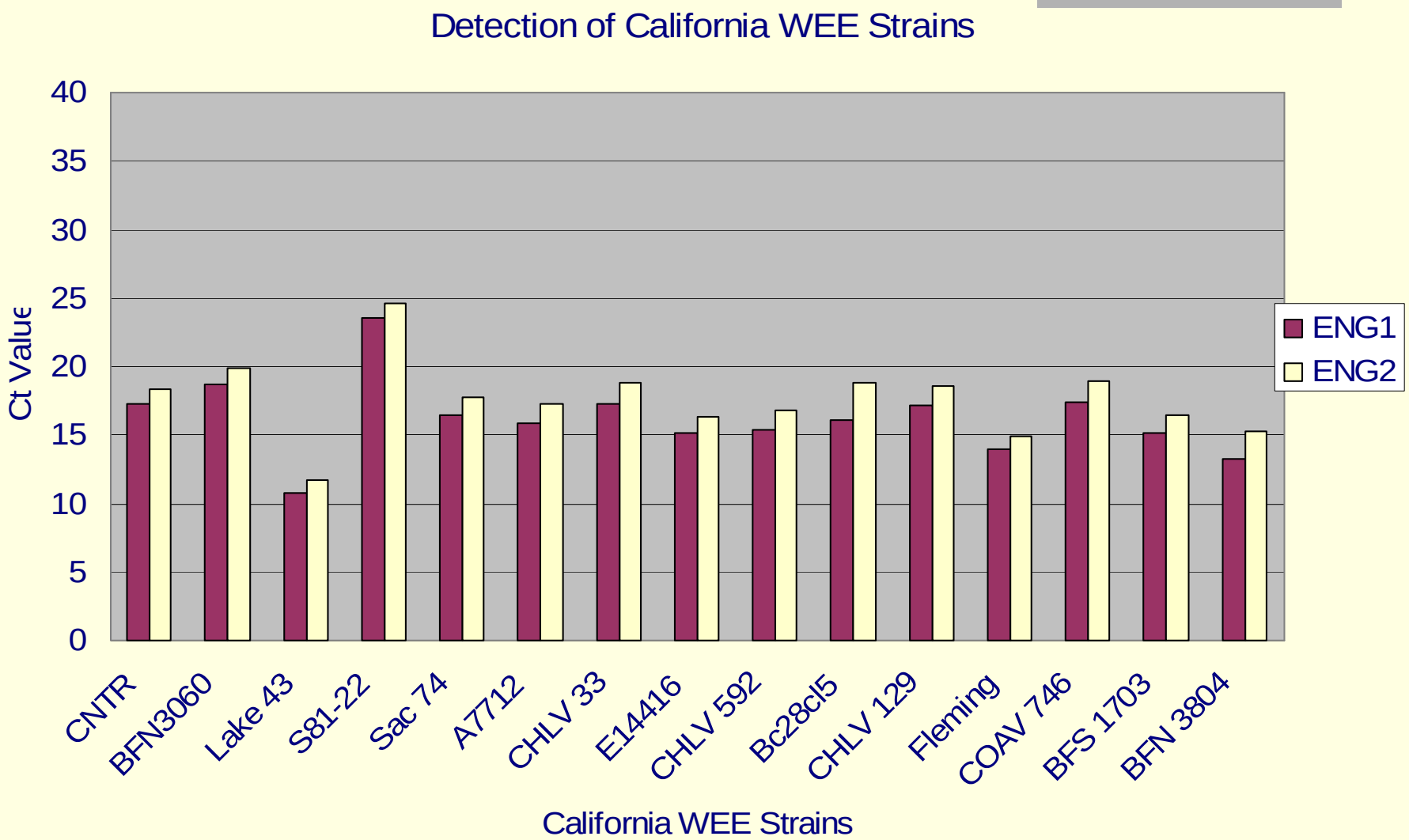
Molecular Surveillance:

SLEV Primer Design for California strains

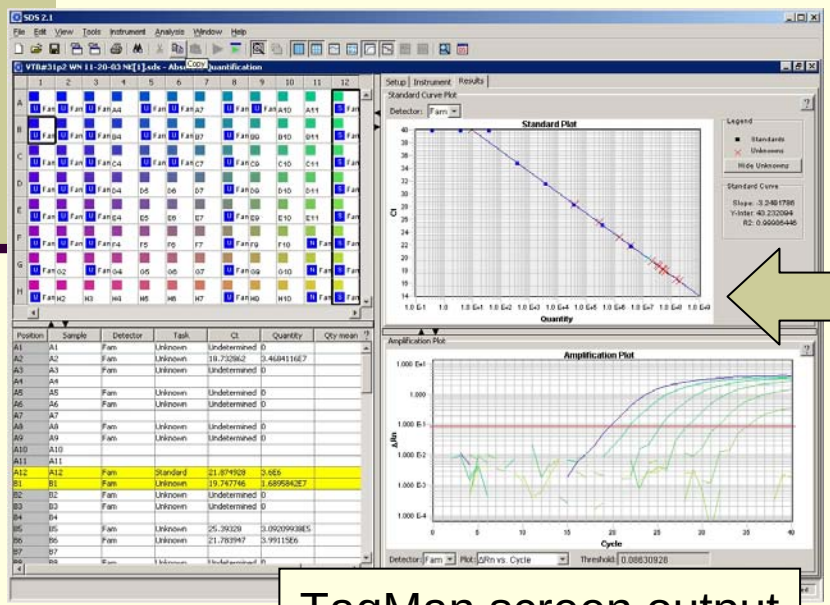


Molecular Surveillance:

WEEV Primer Design for California strains



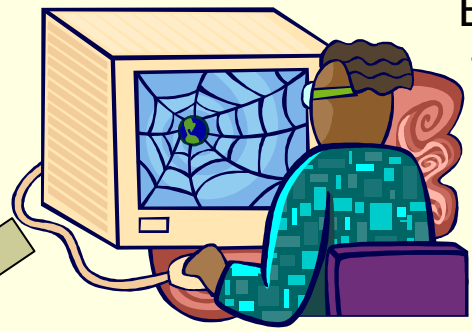
Semi automated molecular diagnostic system



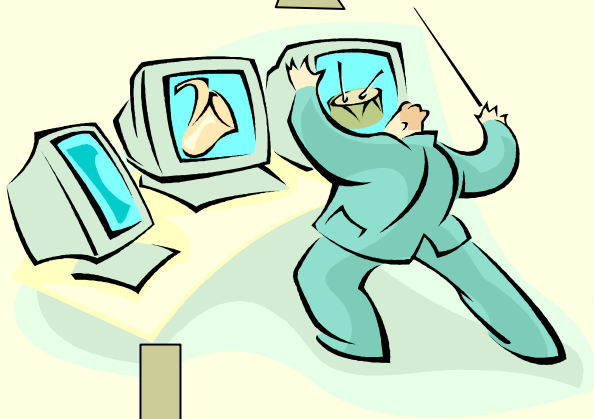
Data flow

MVCAC agencies

Various methods
Packing lists



Barbara enters test results



Bruce, Chris
Steve – central server

Historical database

Arbovirus bulletins

Interactive maps Semi-automatically updated

Comparison of in situ EIA and multiplex RT-PCR

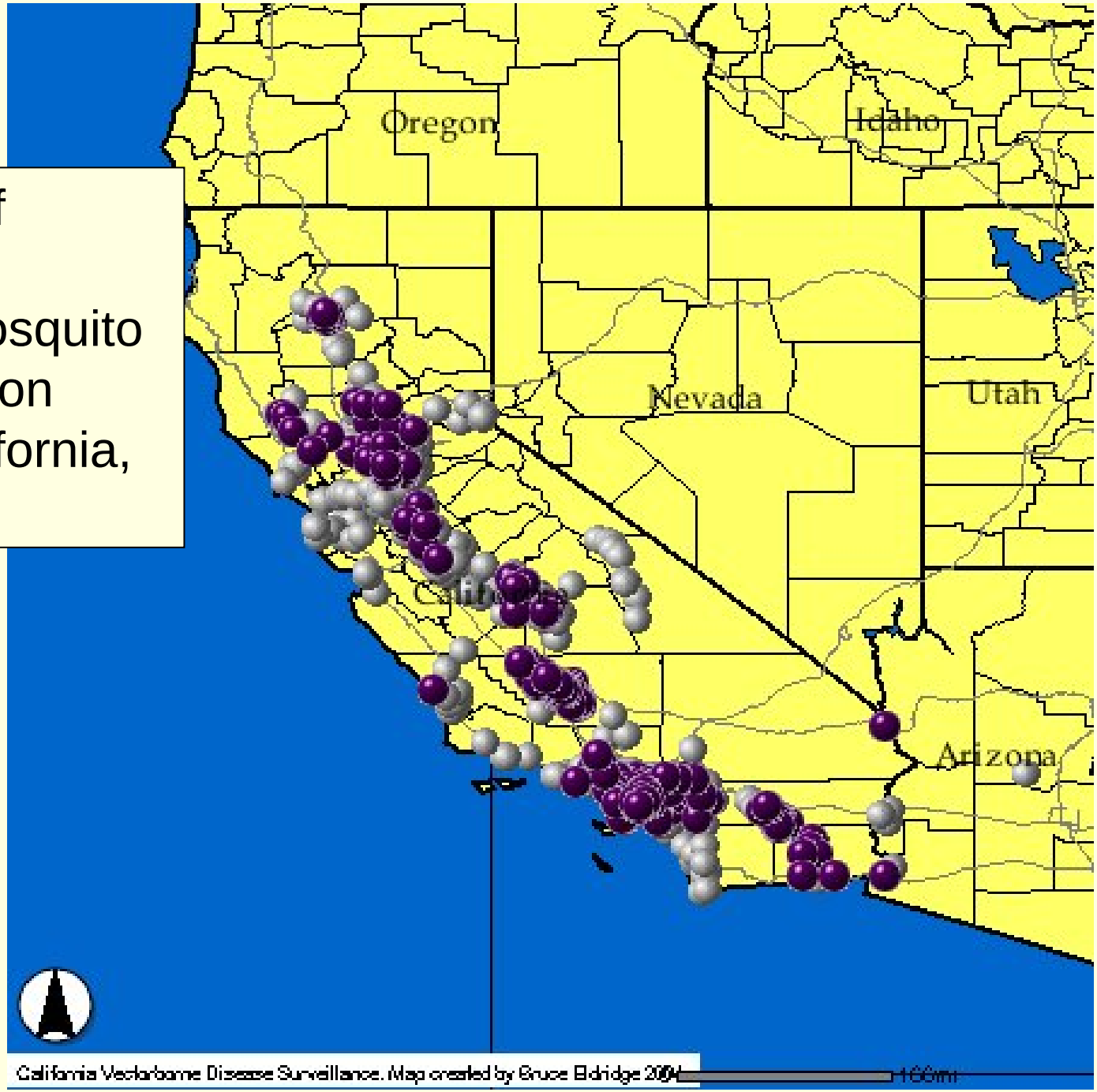
		Multi + single plex RT-PCR		
		Pos	Neg	Total
in situ EIA	Pos	32	2	34
	Neg	5	317	322
	Total	37	319	356
		Sensitivity	86%	
		Accuracy	98%	

Data: 2004 Arbo bull.# 8 -10, GRLA & COAV

Total species, pools, mosquitoes and WNV positives, California, 24 Nov 04

Genus	Species	Pools	Total	WN pos
Aedes	2	108	3,595	0
Anopheles	4	310	9,599	1
Coquilletidia	1	1	8	0
Culiseta	2	473	11,194	0
Culex	8	13,114	501,387	1,131
Ochlerotatus	6	593	25,224	3
Psorophora	1	3	88	0
Total	24	14,602	551,095	1,135

Locations of positive and negative mosquito pool collection sites in California, 2004



Data utilization: MIR

- Definition: Minimum infection rate
- Calculation [simple method]

  MIR per 1,000 = (pos pools/total tested)*1,000

  Formula adequate if infection rate is low and pool sizes similar [i.e., most are 50/pool].

  Note: range with pool size of 50 is 1-20/1,000
- MIRs calculated by district by C Barker [CVEC] and emailed weekly to MVCAC and DHS agencies
- Other Calculations

  CDC has Exel spreadsheet add-in to do calculations using several methods

  [http://www.cdc.gov/ncidod/dvbid/westnile/software.htm]

MIRs in the risk assessment model

Normal

Emergency Planning

Epidemic

Risk Level	MIR per 1,000 [*Cx. tarsalis* + *Cx. pipiens*]
1	0
2	0.1 – 1.0
3	1.1 – 2.0
4	2.1 – 5.0
5	>5.0*

[*MIRs: GRLA>8.3 & KERN >5.5 per 1,000 from Apr-Sep 2004]

Sensitivity of assays for WNV

Testing method	Sensitivity*
Singleplex RT-PCR	< 1
Mutiplex RT-PCR	>1-5
In situ EIA**	>5-10
RAMP	>1,000
VecTest	>10,000

Cx. tarsalis body titers average <10,000 PFU during first collection opportunity so most positive 1-par females VecTest negative

* Infectious viral particles [PFU] per ml

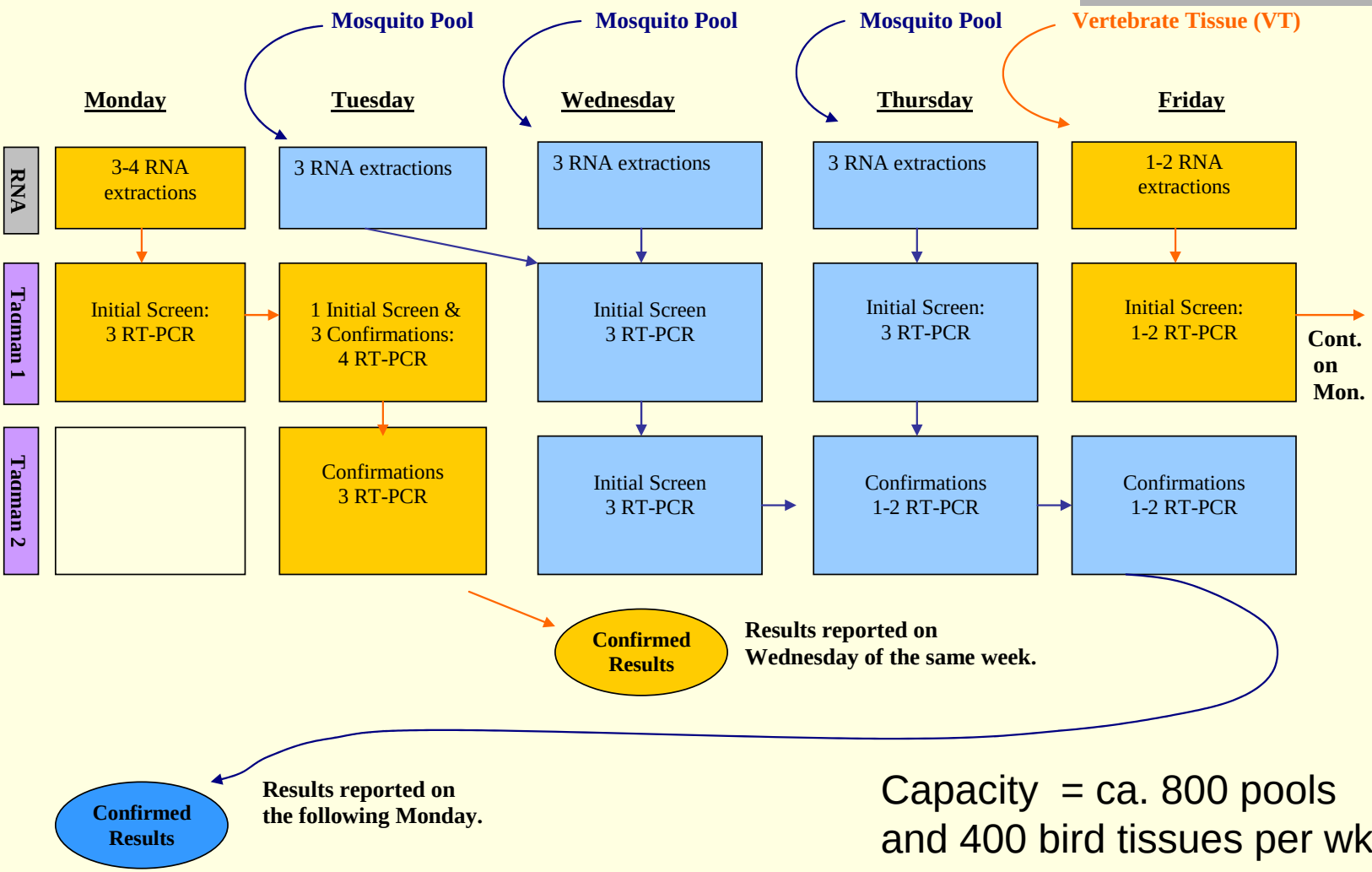
** Viral growth in Vero cells and then Ag detection

Data from Green et al.

Surveillance program designed to take advantage of testing schedule at CVEC

	Mon	Tue	Wed	Thu	Fri
Wk-1	trap mosquitoes and freeze pools				
Wk-2	Ship overnight	Arrive at CVEC	Grind & extract RNA	RT-PCR	Report

2005 Projected Peak Season: Two Taqmen / 8 RT-PCR per day maximum



Capacity = ca. 800 pools and 400 bird tissues per wk